Development and Validation of a Rp-Hplc-Pda Method for Simultaneous Determination of Linagliptin and Metformin in Pharmaceutical Dosage Form

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***Abstract*—A simple, precise, and accurate reverse-phase high-performance liquid chromatography with photodiode array detection (RP-HPLC-PDA) method was developed and validated for the simultaneous estimation of Linagliptin and Metformin in pharmaceutical dosage forms. The chromatographic separation was achieved using an X-Bridge C18 column (4.6 × 150 mm, 5 µm) at a temperature of 35°C. The mobile phase consisted of Acetone and Methanol in a ratio of 65:35 (v/v), delivered at a flow rate of 1 mL/min. Detection was carried out at a wavelength of 220 nm, with an injection volume of 10 µL and a run time of 7 minutes. The method resulted in clear resolution and well-defined peaks for both drugs with acceptable retention times. Validation of the method was performed in accordance with ICH guidelines, and the results demonstrated excellent linearity, accuracy, precision, specificity, and robustness. The developed method is suitable for routine quality control analysis of Linagliptin and Metformin in combined pharmaceutical formulations due to its simplicity, speed, and reproducibility.**

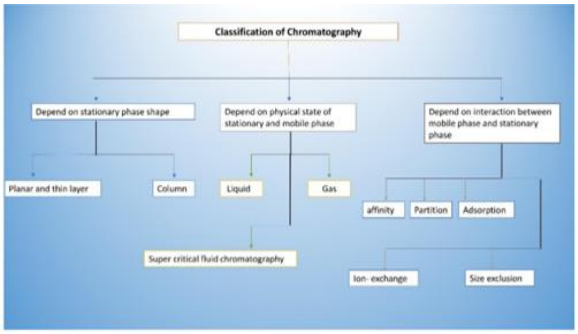
***Index Terms*—RP-HPLC, Linagliptin and Metformin, X-Bridge C18 column, simultaneous estimation, validation.**

I. INTRODUCTION

Chromatography means color-writing and the more specific definition is, it is a physical process of separation at which a mixture of compounds can be separated and isolated, purified into different molecules that depend on different distribution rates depending on Solubility Affinity (if polar or non-polar molecules) Interaction with fixed material (the stationary phase, which we will define later), the components in the mixture are dispersed between two phases, the stationary phase, and the mobile phase, that moves at various speeds in a specified direction.[1] It is known that Michael Tswett, the Russian botanist in 1901 observe that chlorophyll pigments are separated into different colored components when he uses a column containing CaCO3 and moves its mixture on it .so, he is named the founder and father of chromatography, Archer John Porter Martin and Richard Laurence Millington in 1952 won Nobel Prize in Chemistry for their work and efforts in developed many- based separation techniques like partition (liquid-liquid chromatography). [2]

Principle of chromatography

Molecules in a mixture are fixed on the surface of the stationary face, and the mobile phase will be injected to pass on the solid phase carrying the mixture to be separated. Molecular features linked to adsorption (liquid-solid), partition (liquid-solid), and affinity or variations among their molecular weights are the most important factors effective on this separation process. For these differences, some components in the mixture take a long time on the stationary phase and move slowly through the chromatographic system, while others leave the system faster[3].



Chromatographic techniques Figure 1. A graphical diagram shows the classification of chromatography according to three different parameters to form many and vary techniques.

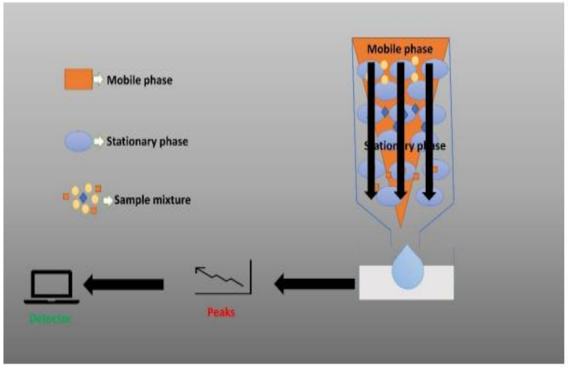
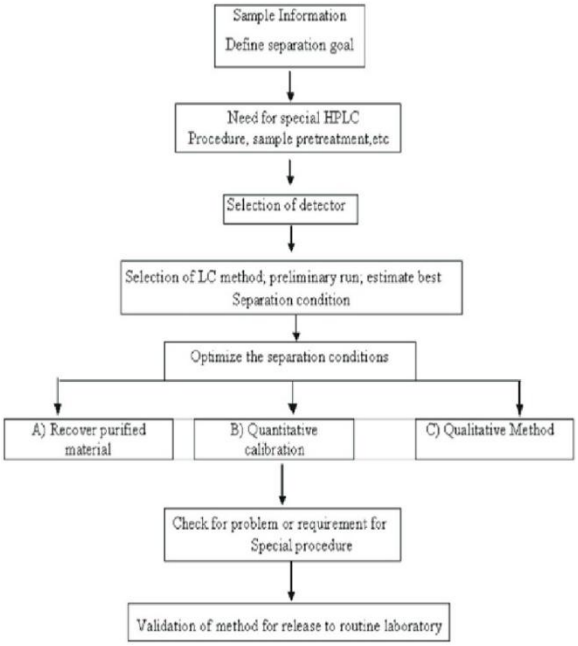


Figure 2. Shows the main procedure for the chromatographic separation method. Column Chromatography Analytical method development using RP-HPLC

Methods of analysis are routinely developed, improved, validated, collaboratively studied and applied. Compilations of these developed methods then appear in large compendia such as USP, BP and IP, etc. In most cases as desired separation can be achieved easily with only a few experiments. In other cases a considerable amount of experimentation may be needed. However, a good method development strategy should require only as many experimental runs as are necessary to achieve the desired final result(s). The development of a method of analysis is usually based on prior art or existing literature using almost the same or similar experimentation. The development of any new or improved method usually tailors existing approaches and instrumentation to the current analyte, as well as to the final need or requirement of the method[4-6].

Method development usually requires selecting the method requirements and deciding on what type of instrumentation to utilize and why. In the HPLC method development stage, decisions regarding choice of column, mobile phase, detectors, and method quantitation must be considered. So development involves a consideration of all the parameters pertaining to any method.

Therefore, development of a new HPLC method involves selection of best mobile phase, best detector, best column, column length, stationary phase and best internal diameter for the column.[6] The analytical strategy for HPLC method development contains a number of steps, as shown in figure[7-8].



II.MATERIALS AND METHODS

The required materials for the process development are procured from the Sura Pharma labs. Linagliptin and Metformin from Sura Pharma labs. Water and Methanol for HPLC LICHROSOLV from(MERCK), Acetone for HPLC Merck.

HPLC METHOD DEVELOPMENT:

Preparation of standard solution:

Accurately weigh and transfer 10 mg of Linagliptin and Metformin working standard into a 10mlof clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removalof air completely and make volume up to the mark with the same Methanol.Further pipette 2.45ml of the above Linagliptin and 0.25ml of the Metformin stock solutions intoa 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure:

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization:

Initially the mobile phase tried was Methanol: Acetone, Acetonitrile: Water with varying proportions. Finally, the mobile phase was optimized to Acetone and Methanol in proportion65:35 v/v respectively.

Optimization of Column:

The method was performed with various columns like C18 column, Phenomenex Luna, Xterra. X- bridge column C18 (4.6 x 150mm, 5mm) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Validation

Preparation of mobile phase:

Accurately measured 650ml (65%) of Acetone and 350ml of Methanol (35%) were mixed and degassed in a digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

The Mobile phase was used as the diluent.

Validation parameters

System suitability

Accurately weigh and transfer 10 mg of Linagliptin and Metformin working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette 2.45ml of the above Linagliptin and 0.25ml of the Metformin stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure:

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Preparation Of Drug Solutions For Linearity:

Accurately weigh and transfer 10 mg of Linagliptin and Metformin working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I to V: Pipette out 0.75ml(75ppm) ,Pipette out 1.5ml(150ppm), Pipette out 2.25ml(225ppm), Pipette out 3.0ml(300ppm), Pipette out 3.75ml(375ppm) of Linagliptin and 0.15ml (15ppm), 0.3ml(30ppm), 0.45ml(45ppm), 0.6ml(60ppm), 0.75ppm(75ppm) of metformin stocksolutions in to a 10ml of volumetric flask and dilute the solution. Performes sonication for 10minutes. Inject each level into the chromatographic system and measure the peak area.Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

Precision Repeatability: The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Intermediate Precision: To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure:

On day1 and day2 the standard solution were injected for six times and measured the area for all six injections inHPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Accuracy:

For preparation of 50-150%Standard stock solution:

Accurately weigh and transfer 10 mg of Linagliptin and Metformin working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 1.12ml(50%), 2.45ml(100%), 3.37ml (150%) of Linagliptin and 0.225ml(50%), 0.25ml(100%),0.675ml(150%) of metformin stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent. Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Linagliptin and Metformin and calculate the individual recovery and mean recovery values.

Robustness:

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results.

Effect of Variation of flow conditions:

The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

Effect of Variation of mobile phase organic composition:

The sample was analyzed by variation of mobile phase i.e. Acetone: Methanol was taken in the ratio and 60:40, 70:30 instead of 65:35, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

III. RESULTS AND DISCUSSION

SPECIFICITY

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. Analytical method was tested for specificity to measure accurately quantitate Linagliptin and Metformin in drug product.

LINEARITY

CHROMATOGRAPHIC DATA FOR LINEARITY STUDY:

The response linearity of Linagliptin and metformin were verified Correlation Coefficient (r) were 0.99, 0.99 and the intercept is 29909 and 454.81 respectively. These values meet the validation criteria.

Precision:

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

Reeatability:

Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

Limit Of Detection

Result: Linagliptin:12.5µg/ml Metformin:3.7µg/ml.

Quantitation limit

Result: Linagliptin:38.1µg/ml Metformin:11.4µg/ml.

IV. CONCLUSION

The method showed good resolution between the peaks of Linagliptin and Metformin, with acceptable system suitability parameters including retention time, peak symmetry, and theoretical plates. The developed RP-HPLC method is simple, accurate, and reliable for the simultaneous estimation of Linagliptin and Metformin in pure drug and combined pharmaceutical formulations. The optimized chromatographic conditions ensure efficient separation with short run time, making the method suitable for high-throughput analysis in quality control laboratories. Its validation as per ICH guidelines further confirms its applicability for routine use in pharmaceutical analysis. . In conclusion, the developed RP-HPLC-PDA method is reliable and efficient for routine analysis of Linagliptin and Metformin in combined pharmaceutical formulations, making it suitable for use in quality control and routine laboratory applications.

Table-1: Peak Results for Optimized Condition of standard and sample

S. No	**Name**	**RT**	**AREA**	**HEIGHT**	**USP Tailing**	**USP Plate count**	**USP Resol ution**
1	Linagliptin	3.202	2391746	39726	1.2	9028	
2	Metformin	5.463	194627	8497	1.1	7398	7.4
3	Linagliptin	3.213	2381649	391846	1.2	9472	
4	Metformin	5.478	191057	8104	1.1	8936	7.5

Table-2:Results of system suitability for Linagliptin and Metformin

Drug	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing	
Linagliptin	Injection-1	3.200	2391746	394171	8952	1.2	Mean area: 2387261 Std. Dev: 4363.771 % RSD:
	Injection-2	3.248	2391647	381946	9561	1.2	
	Injection-3	3.299	2381647	391746	6572	1.2	
	Injection-4	3.297	2385631	386562	6452	1.2	
	Injection-5	3.297	2385635	389164	7452	1.2	
Metformin	Injection-1	5.413	198362	7917	5272	1.1	Mean area: 198001.4 Std.Dev: 535.1774 % RSD: 0.27029
	Injection-2	5.484	197486	7486	6291	1.1	
	Injection-3	5.405	198354	7859	6184	1.1	
	Injection-4	5.405	197352	7926	7145	1.1	
	Injection-5	5.409	198453	7946	6946	1.1	

Table-3 Peak results for assay standard and Sample

	Drug name	RT	Area	Height	USP Tailing	USP Plate Count	Resolution
Standard	Linagliptin	3.211	2397162	397161	1.2	9472	
	Metformin	5.414	198462	7811	1.1	8492	7.49
	Linagliptin	3.222	2394721	389173	1.2	9745	
	Metformin	5.453	198472	8193	1.1	8916	7.52
	Linagliptin	3.254	2389461	391723	1.2	8917	
	Metformin	5.424	198735	7972	1.1	9372	7.44
Sample	Linagliptin	3.297	2391741	381612	1.2	9472	
	Metformin	5.435	198641	8174	1.1	9284	5.435
	Linagliptin	3.294	2389166	391746	1.2	8927	
	Metformin	5.417	196547	8942	1.1	8974	5.417
	Linagliptin	3.295	2361731	381634	1.2	9017	
	Metformin	5.434	194027	7294	1.1	9017	5.434

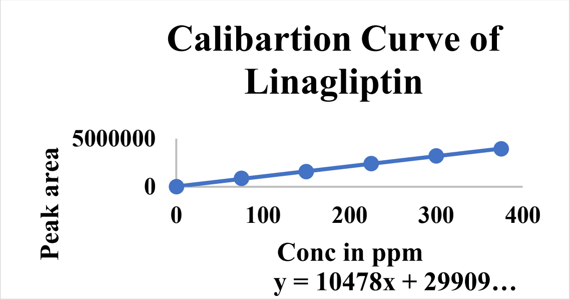
Table -4 Results of repeatability for Linagliptin &Metformin

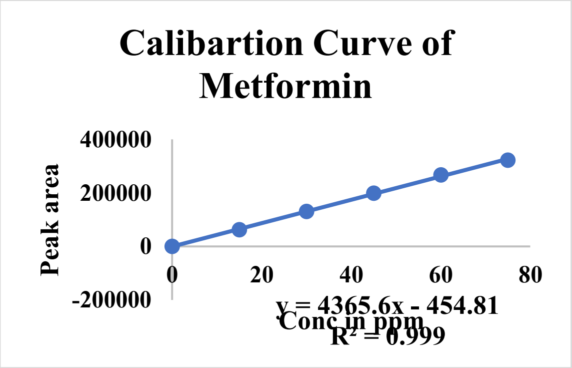
Drug	Name	Rt	Area	Height	USP plate count	USP Tailing	
Linagliptin	Inj-1	3.213	2397164	381741	8155	1.2	Mean 2378830 Std. Dev 14958% RSD 0.628797
	Inj-2	3.253	2391741	371742	9174	1.2	
	Inj-3	3.297	2371846	391746	7154	1.2	
	Inj-4	3.215	2361748	391847	9917	1.2	
	Inj-5	3.254	2371649	384622	9247	1.2	
Metformin	Inj-1	5.441	198464	7291	6274	1.1	Mean 196335.4 Std. Dev
	Inj-2	5.442	193643	7219	6592	1.1	
	Inj-3	5.409	196462	7194	6028	1.1	

	Inj-4	5.520	194644	8174	6927	1.1	2190.191% RSD 1.115536
	Inj-5	5.424	198464	8653	5920	1.1	

Chromatographic data for linearity study:

Drug	Concentration µg/ml	Average Peak Area
Linagliptin	00	00
	75	859889
	150	1583641
	225	2395378
	300	3185089
	375	3943725
Metformin	00	00
	15	61953
	30	130213
	45	198697
	60	267002
	75	321658





Intermediate precision:

Table -5 Results of Intermediate precision for Linagliptin & Metformin

Drug	Day	Name	Rt	Area	Height	USP plate count	USP Tailing	
Linagliptin	Day1	Injection-1	3.211	2389572	395275	9375	1.2	Mean area: 2363409 Std. Dev 39730.83 % RSD 1.681082
		Injection-2	3.211	2391847	392175	9275	1.2	
		Injection-3	3.210	2319472	312947	8265	1.2	
		Injection-4	3.212	2306842	310585	6254	1.2	
		Injection-5	3.211	2375972	310694	9028	1.2	
		Injection-6	3.297	2396746	358373	8928	1.2	
Metformin		Injection-1	5.411	197284	7194	8264	1.2	Mean area: 197281.5 Std. Dev 1466.354 % RSD 0.74328
		Injection-2	5.410	197849	7294	9174	1.2	
		Injection-3	5.420	196572	7147	9164	1.2	
		Injection-4	5.423	195028	7927	9733	1.2	
		Injection-5	5.419	199474	8238	9194	1.2	
		Injection-6	5.409	197482	7638	8973	1.2	
Linagliptin	Day2	Injection-1	3.211	2389562	391741	9264	1.2	Mean area: 2384353 Std. Dev
		Injection-2	3.233	2381654	391047	9746	1.2	
		Injection-3	3.244	2381946	391748	9816	1.2	
		Injection-4	3.297	2391741	391746	9917	1.2	

		Injection-5	3.297	2386452	381641	9742	1.2	6183.339 % RSD 0.25933
		Injection-6	3.202	2374763	381645	9017	1.2	
Metformin		Injection-1	5.411	197486	7582	6272	1.1	Mean area: 197104.2 Std. Dev 903.542 RSD 0.458408
		Injection-2	5.410	197486	7184	6174	1.1	
		Injection-3	5.420	196746	7456	5184	1.1	
		Injection-4	5.405	195862	7814	6194	1.1	
		Injection-5	5.409	196582	7194	6292	1.1	
		Injection-6	5.463	198463	7745	6191	1.1	

ACCURACY:

Table -6 Results of Accuracy at different concentrations (50%, 100%, and 150%)

Concentration of drug(%)	Drug	Rt	Area	Height	USP Tailing	USP plate count	Injection
50%	Linagliptin	3.297	1214719	156568	1.1	6353	1
	Metformin	5.443	98627	7462	1.1	7833	1
	Linagliptin	3.297	1218462	164774	1.2	8632	2
	Metformin	5.453	98634	7642	1.1	8264	2
	Linagliptin	3.200	1218472	159664	1.1	7554	3
	Metformin	5.464	98535	7814	1.1	7642	3
100%	Linagliptin	3.200	2397481	311422	1.1	7462	1
	Metformin	5.459	198371	7112	1.1	8464	1
	Linagliptin	3.202	2397471	316154	1.1	6726	2
	Metformin	5.463	198353	7148	1.1	7364	2
	Linagliptin	3.210	2396471	311973	1.1	5756	3
	Metformin	5.455	198355	7191	1.1	7733	3
150%	Linagliptin	3.204	3512923	311841	1.1	7622	1
	Metformin	5.485	291461	7192	1.2	7464	1
	Linagliptin	3.212	3518846	316452	1.1	8462	2
	Metformin	5.482	291431	7111	1.1	9375	2
	Linagliptin	3.210	3511871	318644	1.1	7483	3
	Metformin	5.403	291645	7125	1.1	6733	3

ROBUSTNESS

Table7- Results of Robustness of Linagliptin & Metformin

Drug	Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Linagliptin	Actual Flow rate of 1.0 mL/min	2391746	3.202	9028	1.2
	Less Flow rate of 0.9 mL/min	2371831	3.639	7381	1.2
	More Flow rate of 1.1 mL/min	2218319	2.859	9311	1.1
	Less organic phase	2294821	3.460	7462	1.2
	More organic phase	2394811	3.022	6817	1.1

Metformin	Actual Flow rate of 1.1mL/min	194627	5.463	7398	1.1
	Less Flow rate of 0.9mL/min	183738	6.250	6883	1.1
	More Flow rate of 0.8mL/min	198373	4.863	9917	1.2
	Less organic phase	178471	6.196	8372	1.1

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